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Experimental paper

The acute effects of acetate-balanced colloid and crystalloid resuscitation on renal oxygenation in a rat model of hemorrhagic shock☆

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ABSTRACT

Introduction: Fluid resuscitation therapy is the initial step of treatment for hemorrhagic shock. In the present study we aimed to investigate the acute effects of acetate-balanced colloid and crystalloid resuscitation on renal oxygenation in a rat model of hemorrhagic shock. We hypothesized that acetate-balanced solutions would be superior in correcting impaired renal perfusion and oxygenation after severe hemorrhage compared to unbalanced solutions.
Methods: In anesthetized, mechanically ventilated rats, hemorrhagic shock was induced by withdrawing blood from the femoral artery until mean arterial pressure (MAP) was reduced to 30 mmHg. One hour later, animals were resuscitated with either hydroxyethyl starch (HES, 130/0.42 kDa) dissolved in saline (HES-NaCl; $n = 6$) or a acetate-balanced Ringer's solution (HES-RA; $n = 6$), as well as with acetated Ringer's solution (RA; $n = 6$) or 0.9% NaCl alone (NaCl; $n = 6$) until a target MAP of 80 mmHg was reached. Oxygen tension in the renal cortex ($C\mu PO_2$), outer medulla ($M\mu PO_2$), and renal vein were measured using phosphorimetry.
Results: Hemorrhagic shock (MAP = 30 mmHg) significantly decreased renal oxygenation and oxygen consumption. Restoring the MAP to 80 mmHg required 24.8 ± 1.7 ml of NaCl, 21.7 ± 1.4 ml of RA, 5.9 ± 0.5 ml of HES-NaCl ($p < 0.05$ vs. NaCl and RA), and 6.0 ± 0.4 ml of HES-RA ($p < 0.05$ vs. NaCl and RA). NaCl, RA, and HES-NaCl resuscitation led to hyperchloremic acidosis, while HES-RA resuscitation did not. Only HES-RA resuscitation could restore renal blood flow back to ~85% of baseline level (from 1.9 ± 0.1 ml/min during shock to 5.1 ml ± 0.2 ml/min 60 min after HES-RA resuscitation) which was associated with an improved renal oxygenation ($C\mu PO_2$ increased from 24 ± 2 mmHg during shock to 50 ± 2 mmHg 60 min after HES-RA resuscitation) albeit not to baseline level. At the end of the protocol, creatinine clearance was decreased in all groups with no differences between the different resuscitation groups.
Conclusion: While resuscitation with the NaCl and RA (crystalloid solutions) and the HES-NaCl (unbalanced colloid solution) led to hyperchloremic acidosis, resuscitation with the HES-RA (acetate-balanced colloid solution) did not. The HES-RA was furthermore the only fluid restoring renal blood flow back to ~85% of baseline level and most prominently improved renal microvascular oxygenation.

1. Introduction

Hemorrhagic shock is the major cause of mortality after major trauma and aggressive fluid resuscitation is often the initial step to restore the circulating intravascular volume to prevent organ hypoperfusion, organ failure, and eventually death.[1,2] Acute renal failure (ARF) is a serious complication contributing to the high mortality in these patients.[35] The use of conventional crystalloid solutions (e.g., isotonic saline) as initial resuscitation fluids is still implemented in emergency departments even though it is known that crystalloid solutions have poor plasma expander capacities and just 20% of the given volume remains contained in the intravascular space.[3] Hence, to restore perfusion, large volumes of crystalloid solutions are required. Additionally, hyperchloremic acidosis is a known risk in patients treated with isotonic saline. Hyperchloremia is suggested to cause afferent renal artery

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vasoconstriction in animal models, possibly leading to kidney dysfunction.[29,38,39] Hydroxyethyl starch (HES) solutions have been used clinically as a colloid solution, and have been shown to have superior plasma expanding capacities compared to traditional crystalloid solutions.[4] However, these HES solutions, in turn, have been suggested to have adverse effects on systemic coagulation properties and are potentially harmful for the kidney.[5] Consequently, new HES solutions (mean molecular weight: 130 kDa, degree of substitution: 0.4; HES 130/0.4) have been developed and have been shown to improve microvascular perfusion and reduce macromolecular leakage.[6–9]

Although effective in restoring systemic hemodynamic parameters, aggressive (i.e., large volume) fluid resuscitation introduces non-physiologic levels of plasma ions which depress microvascular function and organ perfusion.[10] The kidney is especially susceptible for this type of injury due to its complex microvascular structure and high oxygen dependency.[11] Over the past few years, research has therefore been focused on balancing fluids to optimally match physiological conditions and thereby prevent microvascular dysfunction and organ hypoperfusion.[12–14] Balanced fluids are suggested to have a more physiological electrolyte composition than conventional saline-based fluids.

In the present study we aimed to investigate the acute effects of acetate-balanced colloid and crystalloid resuscitation on renal oxygenation in a rat model of hemorrhagic shock. To this end, we examined the effects of resuscitation with different fluids: (1) 0.9% NaCl; (2) acetated Ringer's solution; (3) 6% HES with a molecular weight of 130 kDa and molar substitution of 0.4 (HES 130/0.4) in 0.9% NaCl solution (HES-NaCl); and (4) 6% HES with a molecular weight of 130 kDa and molar substitution of 0.42 (HES 130/0.42) in acetate-balanced Ringer's solution (HES-RA). We hypothesized that acetated solutions would have superior resuscitation capacities compared to the other solutions with respect to improving renal oxygenation after severe hemorrhage.

2. Materials and methods

2.1. Animals

All experiments in this study were approved by the institutional Animal Experimentation Committee of the Academic Medical Center of the University of Amsterdam. Care and handling of the animals were in accordance with the guidelines for Institutional and Animal Care and Use Committees. Experiments were performed on 30 Sprague-Dawley rats (Harlan, the Netherlands) with mean ± SD body weight of 350 ± 20 g.

2.2. Surgical preparation

The rats were anesthetized with an intraperitoneal injection of a mixture of 100 mg/kg ketamine (Nimatek®; Eurovet, Bladel, the Netherlands), 0.5 mg/kg medetomidine (Domitor; Pfizer, New York, NY), and 0.05 mg/kg atropine-sulfate (Centrafarm, Etten-Leur, the Netherlands). After tracheotomy, the animals were mechanically ventilated with an FiO_2 of 0.4. Body temperature was maintained at 37 ± 0.5 °C during the entire experiment by external warming. The ventilator settings were adjusted to maintain end-tidal PCO_2 between 30 and 35 mmHg and arterial PCO_2 between 35 and 40 mmHg.

Vessels were cannulated with polyethylene catheters (outer diameter = 0.9 mm; Braun, Melsungen, Germany) for drug and fluid administration and hemodynamic monitoring. A catheter in the right carotid artery was connected to a pressure transducer to monitor mean arterial blood pressure (MAP) and heart rate. The right jugular vein was cannulated for continuous infusion of Ringer Lactate (Baxter, Utrecht, the Netherlands) at a rate of 15 ml/kg/h. The right femoral artery was cannulated for blood shedding and the right femoral vein for fluid resuscitation. The left kidney was exposed, decapsulated, and immobilized in a Lucite kidney cup (K. Effenberger, Pfaffingen, Germany) via a 4 cm incision in the left flank. Renal vessels were carefully separated under preservation of nerves and adrenal gland. A perivascular ultrasonic transient time flow probe was placed around the left renal artery (type 0.7 RB; Transonic Systems Inc., Ithaca, NY, USA) and connected to a flow meter (T206; Transonic Systems Inc.) to continuously measure renal blood flow (RBF). An estimation of the renal vascular resistance (RVR) was made as RVR [dynes s cm^{-5}] = (MAP/RBF) × 100. The left ureter was isolated, ligated and cannulated with a polyethylene catheter for urine collection. The surgical field was covered with a humidified gauze compress throughout the entire experiment to prevent drying of the exposed tissue.

After the surgical protocol (approximately 60 min) one optical fiber was placed 1 mm above the decapsulated kidney and another optical fiber 1 mm above the renal vein to measure oxygenation using a phosphorescence lifetime technique. A small piece of aluminum foil was placed on the dorsal site of the renal vein to prevent contribution of underlying tissue to the phosphorescence signal in the venous PO_2 measurement. Oxyphor G2 (a two-layer glutamate dendrimer of tetra-(4-carboxy-phenyl) benzoporphyrin; Oxygen Enterprises Ltd., Philadelphia, PA, USA) was subsequently infused (6 mg/kg IV over 5 min) followed by a 30 min stabilization period. A short description of the phosphorescence quenching method is given below and a more detailed description of the technology has been previously described.[15]

2.3. Experimental protocol

After stabilization, the animals in experimental groups were bled by the left femoral artery catheter at a rate of 1 ml/min using a syringe pump (Harvard 33 syringe pump; Harvard Apparatus, South Natick, MA) until a MAP of 30 mmHg was reached which was maintained for 1 h by re-infusing or withdrawing blood. Coagulation of the shed blood was prevented by adding 200 UI of heparin in the syringe.

At the end of the hemorrhage phase, the animals were randomized into 5 groups for resuscitation until a target MAP of 80 mmHg was reached with: (1) 0.9% NaCl (NaCl; Na^+ 154 mmol l^{-1}, Cl^- 154 mmol l^{-1}; pH 5.5; $n = 6$); (2) Ringer's Acetate (RA; Na^+ 130 mmol l^{-1}, Cl^- 112 mmol l^{-1}, K^+ 5.4 mmol l^{-1}, Ca^{+2} 0.9 mmol l^{-1}, Mg^{+2} 1.0 mmol l^{-1}, acetate$^-$ 27 mmol l^{-1}; pH = 5.0–7.0; $n = 6$); (3) 6% HES with a molecular weight of 130 kDa and molar substitution of 0.4 (HES 130/0.4) in 0.9% NaCl solution (HES-NaCl; Voluven®, Fresenius Kabi, Bad Homburg, Germany; $n = 6$); or (4) 6% HES with a molecular weight of 130 kDa and molar substitution of 0.42 (HES 130/0.42) in acetate-balanced Ringer's solution (HES-RA; Plasma Volume®, Baxter, Germany; $n = 6$). In addition, sham operated control experiments were performed ($n = 6$).

The experiments were terminated by infusion of 1 ml of 3 M potassium chloride (KCl).

2.4. Blood gas parameters

Arterial blood samples (0.5 ml) were taken from the femoral artery at time points: (1) baseline (BL, $t = 0$ min); (2) after hemorrhagic shock (HS, $t = 60$ min); (3) 15 min after starting resuscitation (R15, $t = 75$ min), and (4) at the end of the protocol (R60, $t = 120$ min).

The blood samples were replaced by the same volume of test solution. The samples were used to determine blood gas parameters (ABL505 blood gas analyzer; Radiometer, Copenhagen, Denmark),

hemoglobin concentration, and hemoglobin oxygen saturation (OSM 3, Radiometer).

2.5. Renal microvascular and venous oxygenation

Microvascular oxygen tension in the renal cortex ($C\mu PO_2$), outer medulla ($M\mu PO_2$), and renal venous oxygen tension ($P_{rv}O_2$) were measured by oxygen-dependent quenching of phosphorescence lifetimes of the systemically infused albumin-targeted (and therefore circulation-confined) phosphorescent dye Oxyphor G2.[15–18] Oxyphor G2 (a two-layer glutamate dendrimer of tetra-(4-carboxy-phenyl) benzoporphyrin) has two excitation peaks ($\lambda_{excitation1}$ = 440 nm, $\lambda_{excitation2}$ = 632 nm) and one emission peak ($\lambda_{emission}$ = 800 nm).[18] These optical properties allow (near) simultaneous lifetime measurements in microcirculation of the kidney cortex and the outer medulla due to different optical penetration depths of the excitation light.[15] For the measurement of renal venous PO_2 ($P_{rv}O_2$), a mono-wavelength phosphorimeter was used.[19] Oxygen measurements based on phosphorescence lifetime techniques rely on the principle that phosphorescence can be quenched by energy transfer to oxygen resulting in shortening of the phosphorescence lifetime. A linear relationship between reciprocal phosphorescence lifetime and oxygen tension (given by the Stern–Volmer relation) allows quantitative measurement of PO_2. Details of the technique have previously been published.[15]

2.6. Renal oxygen delivery and consumption

Arterial oxygen content (AOC) was calculated by $(1.31 \times \text{hemoglobin} \times S_aO_2) + (0.003 \times P_aO_2)$, where S_aO_2 is arterial oxygen saturation and P_aO_2 is arterial partial pressure of oxygen. Renal venous oxygen (RVOC) content was calculated as $(1.31 \times \text{hemoglobin} \times S_{rv}O_2) + (0.003 \times P_{rv}O_2)$, where $S_{rv}O_2$ is venous oxygen saturation and $P_{rv}O_2$ is renal vein partial pressure of oxygen. Renal oxygen delivery was calculated as DO_2 (ml/min) = RBF × AOC. Renal oxygen consumption is calculated as VO_{2ren} (ml/min/g) = RBF × (AOC − RVOC). The renal oxygen extraction ratio was calculated as O_2ER_{ren} (%) = $VO_{2ren}/DO_2 \times 100$.

2.7. Assessment of kidney function

Creatinine clearance ($Clear_{crea}$, [ml/min]) was assessed as an index of the glomerular filtration rate. Calculation of the clearance was done using the standard formula: $Clear_{crea} = (U_{crea} \times V)/P_{crea}$, where U_{crea} is the concentration of creatinine in urine, V is the urine volume per unit time and P_{crea} is the concentration of creatinine in plasma.

Furthermore, all urine samples were analyzed for sodium (Na^+) concentration. The renal energy efficiency for sodium transport (VO_{2ren}/T_{Na^+}) was assessed using the ratio of the total amount of VO_{2ren} over the total amount of sodium reabsorbed (T_{Na^+}, [mmol/min]).

2.8. Statistical analysis

Values are reported as the mean ± SEM. The decay curves of phosphorescence intensity were analyzed using software programed in Labview 6.1 (National Instruments, Austin, TX, USA). Statistical analysis was performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). Two-way ANOVA with a Bonferroni post hoc test was used and a p-value of <0.05 was considered statistically significant.

Table 1
Amount of resuscitation fluid required to increase the mean arterial pressure from 30 to 80 mmHg and the plasma sodium (Na^+) and chloride (Cl^-) concentrations and plasma pH at baseline (BL) and after 60 min of resuscitation (R60).

	BL	R60
Amount of fluid required [ml]		
Time control		
HS control		
0.9% NaCl		24.8 ± 1.7
Ringer's Acetate		21.7 ± 1.4
HES-NaCl		5.9 ± 0.5[N,R]
HES-RA		6.0 ± 0.4[N,R]
Cl^- [mmol l^{-1}]		
Time control	104 ± 1	105 ± 2
HS control	110.4 ± 1.1	119.5 ± 3.2
0.9% NaCl	87.2 ± 5.4	119.6 ± 6.1[T]
Ringer's Acetate	105.6 ± 3.7	110.2 ± 1.7[T]
HES-NaCl	97.6 ± 1.2	112.4 ± 3.5[T]
HES-RA	102.4 ± 4.1	106 ± 3.5
Na^+ [mmol l^{-1}]		
Time control	142 ± 2	143 ± 2
HS control	148.4 ± 2	149 ± 3.1
0.9% NaCl	141.8 ± 1.0	143.2 ± 0.8
Ringer's Acetate	142.3 ± 1.2	144.2 ± 0.5
HES-NaCl	143.2 ± 1.0	143.8 ± 0.6
HES-RA	142.5 ± 0.9	143.8 ± 0.5
pH		
Time control	7.31 ± 0.10	7.30 ± 0.10
HS control	7.35 ± 0.01	7.11 ± 0.02
0.9% NaCl	7.27 ± 0.01	7.10 ± 0.03[T]
Ringer's Acetate	7.31 ± 0.01	7.15 ± 0.01[T]
HES-NaCl	7.29 ± 0.03	7.20 ± 0.02[T]
HES-RA	7.31 ± 0.01	7.26 ± 0.02

[T] p<0.05 vs. time control.
[N] p < 0.05 vs. 0.9% NaCl.
[R] p < 0.05 vs. Ringer's Acetate.

3. Results

3.1. Fluid and electrolyte balance

The amount of fluids given during resuscitation and the plasma chloride and sodium levels and plasma pH are presented in Table 1. Restoring the MAP from 30 mmHg (shock) to 80 mmHg required 24.8 ± 1.7 ml of NaCl, 21.7 ± 1.4 ml of RA, 5.9 ± 0.5 ml of HES-NaCl (p < 0.05 vs. NaCl and RA), and 6.0 ± 0.4 ml of HES-RA (p < 0.05 vs. NaCl and RA). Plasma chloride levels were significantly increased (p < 0.05 vs. time control) after NaCl (119.6 ± 6.1 mmol l^{-1}), RA (110.2 ± 1.7 mmol l^{-1}), and HES-NaCl (112.4 ± 3.5 mmol l^{-1}) resuscitation, but not after HES-RA (106.0 ± 3.5 mmol l^{-1}) resuscitation. Similarly, plasma pH was significantly decreased (p < 0.05 vs. time control) after NaCl (7.10 ± 0.03), RA (7.15 ± 0.01), and HES-NaCl (7.20 ± 0.02) resuscitation, but not after HES-RA (7.26 ± 0.02) resuscitation. Hence, NaCl, RA, and HES-NaCl resuscitation led to hyperchloremic acidosis, while HES-RA resuscitation did not.

3.2. Systemic and renal hemodynamics

Systemic and renal hemodynamic variables are presented in Table 2. The baseline values measured in each group were found to be similar (p > 0.05). In all groups, MAP, RBF decreased during hemorrhage without significant differences between groups.

During resuscitation, MAP was consistently increased in all groups, though the target MAP of 80 mmHg was not successfully maintained after 60 min of resuscitation. In crystalloid treated groups, NaCl and RA, MAP was lower at the end of the protocol (44 ± 4 and 48 ± 3 mmHg, respectively) compared to in the colloid treated groups, HES-NaCl and HES RA (58 ± 5 and 52 ± 3 mmHg, respectively).

Table 2
Mean arterial pressure (MAP), renal blood flow (RBF), and renal vascular resistance (RVR) at baseline (BL), during hemorrhagic shock (HS), and after 15 and 60 min of resuscitation (R15 and R60, respectively).

	BL	HS	R15	R60
MAP [mmHg]				
Time control	102 ± 1	104 ± 2	99 ± 3	105 ± 4
HS control	95 ± 9	30 ± 2	30 ± 3	30 ± 2
0.9% NaCl	102 ± 2	31 ± 1	73 ± 8	44 ± 4
Ringer's Acetate	101 ± 2	33 ± 1	57 ± 2[H]	48 ± 3
HES-NaCl	102 ± 3	31 ± 1	67 ± 6	58 ± 5
HES-RA	101 ± 3	32 ± 2	62 ± 3	52 ± 3
RBF [ml/min]				
Time control	5.6 ± 0.4	5.6 ± 1.1	5.8 ± 0.6	5.5 ± 1.0
HS control	5.4 ± 0.2	1.2 ± 0.2	1.1 ± 0.3	0.9 ± 0.2
0.9% NaCl	5.6 ± 0.3	1.4 ± 0.1	2.9 ± 0.6[H]	2.4 ± 0.5
Ringer's Acetate	5.6 ± 0.4	1.4 ± 0.2	3.8 ± 0.5[H]	3.6 ± 0.4[H]
HES-NaCl	5.5 ± 0.2	1.7 ± 0.3	3.1 ± 0.3[H]	3.4 ± 0.4[H]
HES-RA	5.9 ± 0.2	1.9 ± 0.1	4.9 ± 0.4[H]	5.1 ± 0.2[H,N]
RVR [dyn s s^{-5}]				
Time control	16.4 ± 1.1	15.1 ± 1.5	17.2 ± 1.2	16.9 ± 1.5
HS control	17.5 ± 1.8	24.5 ± 5.2	27.6 ± 5.4	37.7 ± 5.6
0.9% NaCl	18.6 ± 1.5	22.3 ± 1.8	29.3 ± 4.6	21.4 ± 3.5
Ringer's Acetate	18.5 ± 1.5	20.8 ± 1.2	15.8 ± 2.5	14.2 ± 2.0
HES-NaCl	18.6 ± 1.3	19.9 ± 3.0	21.4 ± 3.5	18.7 ± 2.7
HES-RA	17.1 ± 1.0	17.6 ± 0.9	13.1 ± 1.0	10.2 ± 0.5[H,N]

[H] $p < 0.05$ vs. HS control.
[N] $p < 0.05$ vs. 0.9% NaCl.

Resuscitation improved RBF in all groups starting in the early phase of resuscitation ($p < 0.05$). Improvement of RBF after 60 min of resuscitation was most pronounced in the HES-RA group (5.1 ± 0.2 ml/min; 85% of baseline value) and least in 0.9% NaCl group (2.4 ± 0.5 ml/min; 42% of baseline value).

3.3. Renal oxygenation

Renal DO_2, VO_2, $C\mu PO_2$, and $M\mu PO_2$ are presented in Table 3. All these parameters decreased during hemorrhage without significant differences between groups. At the end of resuscitation, DO_2 was improved compared to hemorrhagic shock. This increase was significant in the RA group (0.41 ± 0.07 ml O_2/min) and HES-RA group (0.39 ± 0.06 ml O_2/min) compared to HS control ($p < 0.05$). VO_2, however, could not be increased by fluid resuscitation ($p > 0.05$ vs. HS control).

Resuscitation improved $C\mu PO_2$ and $M\mu PO_2$ albeit not to baseline level. At R60, $C\mu PO_2$ was higher in the HES-RA group compared to other groups and significantly different comparing to the NaCl group ($p < 0.05$).

3.4. Renal function

Creatinine clearance and VO_2/T_{Na^+} are presented in Fig. 1. There were no differences at baseline in creatinine clearance (not shown). During hemorrhagic shock urine production decreased dramatically. In the HS control group, all animals suffered from anuria at the end of the protocol. All groups had a lower creatinine clearance at the end of resuscitation ($p < 0.05$ vs. time control). The NaCl resuscitated group had the lowest creatinine clearance rate at R60. The VO_2/T_{Na^+} was found to be unaffected by fluid resuscitation.

4. Discussion and conclusions

In the present study, we examined the acute effects of acetate-balanced colloid and crystalloid resuscitation on renal oxygenation in a rat model of hemorrhagic shock. We tested the hypothesis that acetate-balanced solutions would be superior in correcting impaired renal perfusion and oxygenation after severe hemorrhage compared to unbalanced solutions. Our main findings were that: (1) hemorrhagic shock was associated with acute decreases in blood pressure, renal perfusion and oxygenation, and urine production; (2) volume replacement therapy with balanced and unbalanced crystalloid and colloid solutions partially corrected these parameters; and (3) the acetate-balanced colloid solution HES-RA was the only resuscitation fluid that could restore renal blood flow back to ~85% of baseline level which was associated with the most prominently improved renal oxygenation.

Hemorrhagic shock is one of the major causes of acute renal failure due to decreased blood pressure and consequent hypoperfusion of the kidney. The presence of acute renal failure significantly increases morbidity and mortality.[40] The first step in the correction of hemorrhage-induced hypotension is aggressive volume replacement therapy[12] which aims to increase the circulating intravascular volume, blood pressure, and organ perfusion.[9,34] However, in contrast to blood, resuscitation fluids have poor oxygen transporting capacity and rheological properties. In addition, the fluids used for volume replacement therapy have been suggested to increase inflammation and disturb homeostasis and the acid–base balance.[41–44] Over time, a variety of colloid and crystalloid solutions has been used, including isotonic saline and saline-based colloid solutions. Although saline-based solutions have been associated with disturbed acid–base balance due to non-physiological electrolyte composition and pH, these yet remain the most popular solutions for volume replacement therapy in peri-operative care.[22–25,29,38,39] With respect to the kidney, saline-based solutions are known to be more frequently associated with hyperchloremic acidosis, due to their high levels of chloride, resulting in renal vascular constriction and decreased renal perfusion.[26a,27,45] This we have confirmed in the present study.

Balanced solutions, in contrast, provide an alternative with optimized physiological composition in terms of sodium, potassium, calcium, magnesium, and chloride levels, and their relative contributions regarding osmolality. Buffers such as acetate, gluconate, pyruvate, and lactate can be used in resuscitation fluids and are converted to bicarbonate in liver and raise the pH of the solution to normal blood pH (7.4). These solutions achieve a physiological acid–base balance with either bicarbonate or metabolizable anions and reduce of the risk of iatrogenic disruptions. In animal models of sepsis it has also been demonstrated that balanced solutions lead to less metabolic acidosis, reduced inflammatory cytokine levels, and longer survival compared to resuscitation with normal saline.[26b,32] Infusion of solutions containing lactate, however, has multiple side effects and, aside from those, lactate buffers require high levels of liver metabolism and oxygen consumption.[28]

In our model, as shown by others, hyperchloremia led to progressive renal vasoconstriction (increased RVR and decreased RBF) and a fall in glomerular filtration rate (decreased creatinine clearance). These phenomena have been shown to be independent of the renal nerve system and to be related to tubular chloride reabsorption and chloride-induced renal vasoconstriction.[29] Increased RVR and decreased creatinine clearance were most pronounced following NaCl resuscitation and were less pronounced in the HES-RA resuscitated group. Furthermore, HES-RA resuscitation was the only regime that could significantly increase renal DO_2. This can be explained by the composition of the different fluids: where 0.9% NaCl has a chloride content of 154 mmol l^{-1}, HES-RA has a chloride content of 112 mmol l^{-1}. It should be pointed out, however, that the improved renal oxygenation in the HES-RA group compared to the other groups is not directly associated with acetate-balancing, per se; rather, it is probably due to less chloride infused in HES-RA in this MAP-targeted resuscitation protocol. Acetate itself does not correct hyperchloremic acidosis, lactic acidosis, and does not protect renal function. However, as HES-RA resuscitation prevented

Table 3
Renal oxygen delivery (DO_2), oxygen consumption (VO_2) and microvascular oxygen tension in the renal cortex ($C\mu pO_2$) and medulla ($M\mu pO_2$) at baseline (BL), during hemorrhagic shock (HS), and after 15 and 60 min of resuscitation (R15 and R60, respectively).

	BL	HS	R15	R60
DO_2 [ml O_2/min]				
Time control	1.30 ± 0.10	1.320 ± 0.15	1.270 ± 0.08	1.40 ± 0.01
HS control	1.420 ± 0.11	0.190 ± 0.05	0.160 ± 0.05	0.130 ± 0.05
0.9% NaCl	1.360 ± 0.09	0.220 ± 0.01	0.330 ± 0.06	0.270 ± 0.06
Ringer's Acetate	1.240 ± 0.03	0.220 ± 0.04	0.480 ± 0.08[H]	0.410 ± 0.07[H]
HES-NaCl	1.390 ± 0.04	0.290 ± 0.09	0.40 ± 0.09[H]	0.350 ± 0.05[H]
HES-RA	1.320 ± 0.08	0.20 ± 0.02	0.530 ± 0.09[H]	0.390 ± 0.06[H]
VO_2 [ml O_2/min/g]				
Time control	0.200 ± 0.02	0.250 ± 0.01	0.240 ± 0.01	0.280 ± 0.02
HS control	0.150 ± 0.08	0.070 ± 0.02	0.070 ± 0.02	0.060 ± 0.02
0.9% NaCl	0.190 ± 0.06	0.070 ± 0.01	0.080 ± 0.02	0.050 ± 0.01
Ringer's Acetate	0.100 ± 0.03	0.060 ± 0.02	0.10 ± 0.04	0.070 ± 0.03
HES-NaCl	0.210 ± 0.03	0.110 ± 0.04	0.090 ± 0.03	0.070 ± 0.02
HES-RA	0.150 ± 0.02	0.070 ± 0.01	0.110 ± 0.03	0.110 ± 0.02
$C\mu pO_2$ [mmHg]				
Time control	800 ± 2	780 ± 2	780 ± 2	760 ± 2
HS control	830 ± 2	280 ± 6	220 ± 4	190 ± 5
0.9% NaCl	850 ± 4	200 ± 2	430 ± 3	330 ± 3
Ringer's Acetate	750 ± 6	210 ± 3	400 ± 3	410 ± 2
HES-NaCl	810 ± 7	270 ± 4	440 ± 3	450 ± 5
HES-RA	850 ± 4	240 ± 2	530 ± 3[R]	500 ± 2[N]
$M\mu pO_2$ [mmHg]				
Time control	670 ± 2	660 ± 1	640 ± 1	640 ± 2
HS control	620 ± 2	190 ± 6	150 ± 2	140 ± 2
0.9% NaCl	670 ± 3	90 ± 1	410 ± 2	300 ± 1
Ringer's Acetate	610 ± 4	110 ± 3	310 ± 3	300 ± 1
HES-NaCl	730 ± 1	160 ± 3	350 ± 2	300 ± 3
HES-RA	670 ± 3	220 ± 3	390 ± 6	300 ± 4

[H] $p < 0.05$ vs. HS control.
[N] $p < 0.05$ vs. 0.9% NaCl.
[R] $p < 0.05$ vs. Ringer's Acetate.

hyperchloremic acidosis, it also led to avoiding microvascular constriction in renal cortex and medulla by which renal oxygenation was improved. Therefore, in essence, this study provided evidence that the excess chloride in resuscitation is toxic and disturbs both the acid–base balance and the organ function.

In this line, metabolic acidosis has been shown to be a common complication in critically ill patients and has been shown to serve as an independent predictor of outcome.[56,57] Furthermore, restricting chloride-rich fluids in intensive care has been shown significantly improve the acid–base status in critically ill patients.[58] However, although several animal studies, including the present study, suggest that hyperchloremic metabolic acidosis leads to renal vasoconstriction and potentially to kidney dysfunction, whether this also occurs in patients remains to be verified.

The results from our study demonstrated once more the need for larger volumes of crystalloids to achieve similar systemic and microcirculatory goals, compared to colloids. Blood pressure increased the first 15–20 min of resuscitation and then gradually declined even though fluid infusion continued. Hence, the volume expansion effect of both crystalloids and colloids were temporary. Nonetheless, significantly lower volumes of colloids were required and the colloid solutions were also more effective in maintaining blood pressure after 1 h of resuscitation. The low efficacy of the crystalloid solutions can be explained by the fact that only 20% of

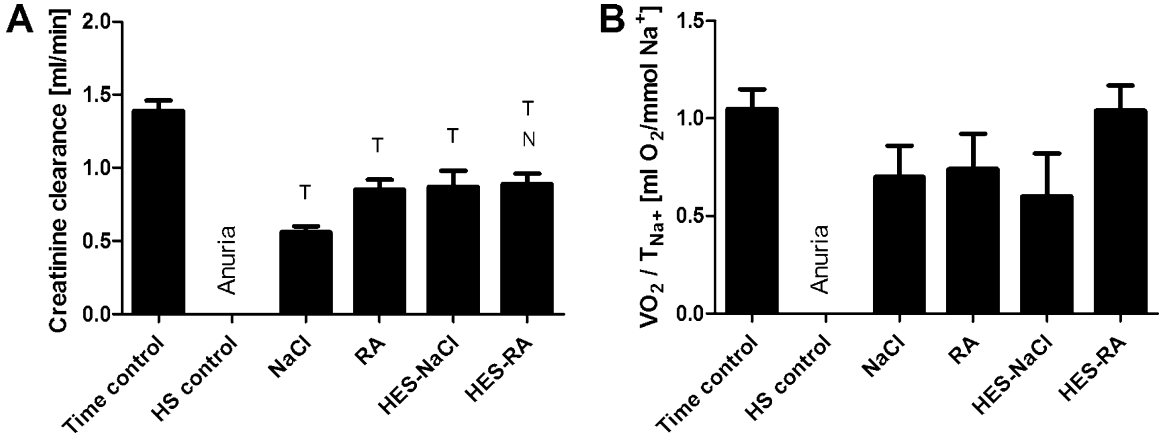

Fig. 1. Creatinine clearance and the ratio of the renal oxygen consumption (VO_2) over the total amount of sodium reabsorbed (T_{Na^+}) after 60 min of resuscitation. [T] $p < 0.05$ vs. time control, [N] $p < 0.05$ vs. 0.9% NaCl.

their volume remains in the vascular lumen and 80% leaks out, leading to tissue edema and consequent impaired tissue oxygenation.

Excessive fluid overload leads to hemodilution which eventually may impair tissue oxygenation. In experimental studies it has been demonstrated that acute isovolemic hemodilution is associated with increases in red blood cell aggregation which triggers endothelium-dependent thrombogenic and pro-inflammatory responses.[52] Animal studies have demonstrated the direct influence of hemodilution on microvascular flow and renal oxygen supply.[53] Johannes et al. have found that the renal microvascular oxygenation drops at very early stages of isovolemic hemodilution. It was also shown that the kidney is particularly vulnerable to decreases in oxygen delivery and that the critical hematocrit associated with a decrease in microvascular oxygenation is much higher for the kidney than for the heart or intestines.[54] This was underscored by a study demonstrating an increased risk of acute kidney injury in cardiopulmonary bypass-associated hemodilution.[55] The reasons for such a high sensitivity to hemodilution could involve endothelial dysfunction with an inflammatory component leading to tissue edema and increase of diffusion distance from microcirculation to the tissue cells.

Although earlier studies suggested negative effects of colloids on microcirculation, there is increasing evidence supporting the opposite.[47] Compared to crystalloid solutions, colloid solutions increase plasma viscosity. Elevating plasma viscosity in extreme hemodilution has been shown to increase microvascular flow through nitric oxide-mediated vasodilation.[48] Others have demonstrated the importance of sufficient blood viscosity with respect to functional capillary density and tissue oxygenation. Hence, during acute hemodilution as occur during aggressive fluid resuscitation, increasing plasma viscosity by administration of colloids may be beneficial for the microcirculation.[49] Indeed, the administration of hyperoncotic and hyperviscous solutions has been shown to be advantageous in hemorrhagic shock due to normalization of colloid osmotic pressure which leads to the recovery of microcirculatory perfusion and oxygenation.[50] Furthermore, Lang et al. described that colloids improved microvascular perfusion and reduced endothelial tissue edema. In contrast, the authors showed that crystalloids leak rapidly into the interstitium, causing endothelial tissue swelling and consequently reducing capillary perfusion and increasing the oxygen diffusion distance.[46] The results from the present study confirm this as microvascular oxygenation in the renal cortex was lower in the crystalloid resuscitated groups compared to the colloid resuscitated groups. This was most marked when comparing the unbalanced crystalloid solution (NaCl) to the acetate-balanced colloid solution (HES-RA) and was also translated into a significantly higher creatinine clearance rate in the HES-RA group compared to the NaCl group.

Our study has, however, some limitations which should be acknowledged. First, translation of the findings in our animal model to clinical scenarios should be done with utmost care. Here, we imitated major hemorrhage by withdrawing blood until mean arterial pressure was decreased to 30 mmHg. Most trauma patients, however, suffer from multiple injuries which may influence their inflammatory state, potentially interfering with the hemorrhage-induced hypovolemia and subsequent treatment. Moreover, this model does not reflect the challenges in treatment of a neurological trauma patient. Nonetheless, our model does demonstrate the efficacy of volume replacement therapy using different types of fluids on renal perfusion and oxygenation after severe hemorrhage. Second, the rather short follow-up period after of hemorrhagic shock and resuscitation does not allow assessment of renal (dys)function and injury in the long-term. Third, blood lactate and base excess levels were not monitored in the experiments so the effects of the tested solutions on these parameters remain to be elucidated.

In conclusion, while resuscitation with the NaCl and RA (crystalloid solutions) and the HES-NaCl (unbalanced colloid solution) led to hyperchloremic acidosis, resuscitation with the HES-RA (acetate-balanced colloid solution) did not. The acetate-balanced colloid solution HES-RA was furthermore the only fluid restoring renal blood flow back to ~85% of baseline level and most prominently improved renal microvascular oxygenation. However, the long-term effects of HES-RA resuscitation on renal function warrants further study.

Conflict of interest statement

All authors declare to have no conflict of interest.

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